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Award Number: DAMD17-98-1-8535

TITLE: Genetic Susceptibility to Prostate Cancer Among Ashkenazi Jews

PRINCIPAL INVESTIGATOR: Harry Ostrer, M.D.
Carole Oddoux, Ph.D.

CONTRACTING ORGANIZATION: New York University Medical Center
New York, New York 10016

REPORT DATE: December 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	December 2002	Annual (15 Sep 01 - 14 Sep 02)

4. TITLE AND SUBTITLE
Genetic Susceptibility to Prostate Cancer Among Ashkenazi Jews

5. FUNDING NUMBERS
DAMD17-98-1-8535

6. AUTHOR(S):
Harry Ostrer, M.D.
Carole Oddoux, Ph.D

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
New York University Medical Center
New York, New York 10016

E-Mail: ostreh01@med.nyu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

This project will test the basic hypothesis that a given microsatellite marker allele occurs with greater frequency among the individuals affected with prostate cancer than among the controls. These studies will take advantage of the fact that two populations of Ashkenazi Jewish men are readily available for a case-control study. The first is a group of men at high heritable risk based on their having early-onset prostate cancer. The second is a group of men at low heritable risk who have no personal or family history of prostate cancer. Thus, we expect to observe predisposition alleles in the men at high risk that are not present in the men at low risk. The predisposition genes are likely to be within chromosomal regions in which loss of heterozygosity has occurred. Because these regions have remained identical by descent since the high-risk mutations occurred, they can be recognized by the presence of specific alleles of microsatellite markers in the high-risk group that are not present in the low-risk group.

14. SUBJECT TERMS:
prostate cancer

15. NUMBER OF PAGES
6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

This study uses several observations about the genetic basis of prostate cancer to enhance the efficiency of identifying susceptibility genes. 1) Prostate cancer is a multi-step genetic disorder in which some of the observed genetic alterations in prostate cancer cells were acquired through the germline. 2) The chromosomal locations of some of these genes can be identified readily in prostate cancer cells on the basis of their demonstrating loss of heterozygosity. 3) Historically, certain populations have been endogamous causing them to have more genetic homogeneity and to have prevalent founder mutations in some of their disease susceptibility genes. As a result of the population's endogamy, short chromosomal regions have remained identical by descent, leading to recognizable associations of the founder mutations with linked marker alleles (*linkage disequilibrium*). Ashkenazi Jews represent such a population.

BODY

Task 1. Subject identification.

Samples from 200 high-risk subjects were identified during the first year. To increase the power of the study, samples from an addition 100 cases were identified during the second year. The medical histories of each of these subjects were reviewed, confirming ethnicity and diagnosis of prostate cancer, and noting family history, age at diagnosis and Gleason score at time of diagnosis. For each subject, tissue blocks were obtained from non-cancerous tissues (usually lymph nodes) and thick (50 micron) sections were cut. DNA was purified from these sections using a protocol optimized in our laboratory and then quantified. To extend the utility of these sections, a technique for whole genome amplification using primer extension preamplification (PEP) was optimized. This technique reproducibly provides approximately 50-fold amplification of the DNA samples. From the pool of low-risk subjects, an additional 200 samples were chosen and added to the 200 samples already obtained for subsequent analysis. These were amplified using PEP for subsequent analysis. Methods of drying down these DNAs in 96-well microtiter plates for subsequent use were developed.

Task 2. Development of markers.

A. Markers from regions associated with loss of heterozygosity (LOH) in prostate cancer will be identified and fluorochrome-labeled primers will be synthesized. We have identified microsatellite markers for each of the following chromosomal regions - 8p21-p22, 10q23-q25, 13q14, 16q22, 17p, 17q21-q22, . We added markers to our analysis. Because of uncertainties about relative map positions, we have refined our selected markers from those which have shown (LOH) in a high proportion of subjects in a single report, to those which show (LOH) in more than one report, or to those whose map positions are known with a high degree of confidence from the GeneMap99 (http://www.ncbi.nlm.nih.gov/GeneMap99) and which are tightly linked to markers that show LOH. In addition, we have added markers for the following chromosomal regions that have shown linkage to prostate cancer susceptibility in families with multiple affected members, 1q24-25, 1q42-43, and Xq27-28 (Smith, et al., 1996, Cooney, et al., 1996, Gronberg, et al., 1997, Xu, et al., 1998, Berthon, et al., 1998)

B. Standard PCR conditions will be developed for each of these markers. The primer sequences for each of these markers was identified using standard databases (http://www.gdb.org). The predicted sizes of the PCR product alleles were noted and markers yielding products of different predicted sizes were grouped and labeled with one of three different fluorescent dyes (tet, fam, hex). The net effect of this grouping is that multiple markers can either be amplified simultaneous and/or pooled from separate amplifications to minimize the number of electrophoretic runs. Procedures for pooling separate amplification reactions have been optimized.

The methods for multiplex analysis were developed to enhance the throughput of marker analysis. For each fluorochome, three markers (small, medium, and large) were coamplified in the same well. In turn, these multiplex amplifications for each of the fluorochomes were pooled for subsequent fragment length analysis. This led the analysis of up to 9 markers simultaneously in a single run.

Task 3. Data analysis

Data analysis was performed for ~30% of the controls. Data analysis was delayed by turnover in technical staff and recurring technical issues with the major analytical instruments (ABI 310 DNA analyzers). These have been remedied.

KEY RESEARCH ACCOMPLISHMENTS:

Development of DNA databases from cases and controls for genomic analysis.

Development of high-quality, reproducible methods for microsatellite typing

Development of high-quality, reproducible methods for whole genome amplification

REPORTABLE OUTCOMES:

Proposal, "Genetic Susceptibility to Prostate Cancer in the Netherlands Cohort Study," (PC99-1496) was funded by USARMC.

Proposal, "Mentorship Program in Prostate Cancer Genetics" K24 (CA85326-01A1), was funded.

CONCLUSIONS

This works demonstrates the feasibility for high-throughput multiplex microsatellite marker analysis and the feasibility for extending small samples of DNA 50-fold for genetic analysis. It creates the foundations for the analyses that will be performed in the remainder of this study.

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